A

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QAAKNTISEF KKVRADTWKF DRASFNTEEL EDLEGVLWRS YYA

Figure S1 (A) Amino acid sequence of Blm3 as deduced from the BLM3-YFL006 gene of yeast Saccharomyces cerevisiae. The putative intergenic region between BLM3 and YFL006 was sequenced. BLM3 was confirmed to be in frame with YFL006 (Yeast Proteome Database). The N-terminal peptide obtained by Edman degradation and tryptic peptides from Blm3 are underlined. The putative nuclear localization sequence is highlighted in grey. The protein kinase phosphorylation site is framed (Ficarro et al. (2002). Phosphoproteome analysis by mass spectrometry and its application to Saccharomyces cerevisiae. Nat. Biotechnol.

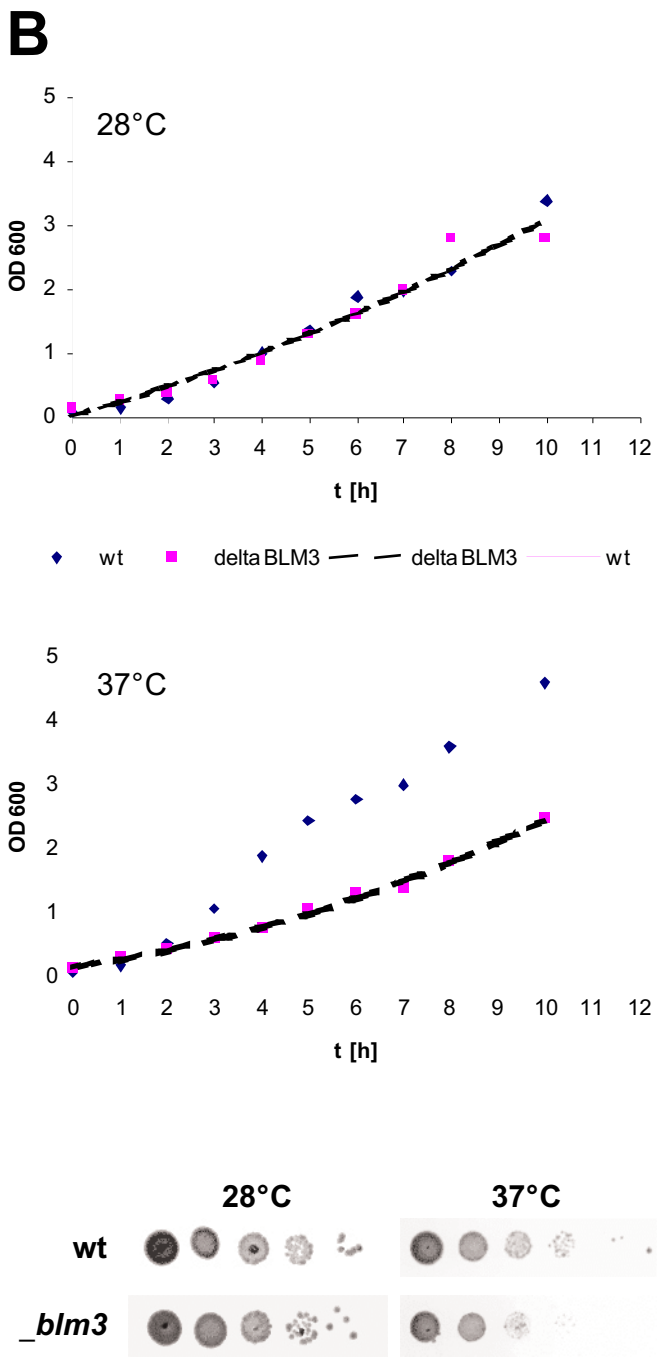

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Figure S1B

(B) The blm3D null strain is temperature sensitive. Growth curves of blm3D compared to isogenic wild type cells in YPD at 28°C and 37°C. Spot assays of blm3D compared to isogenic wild type cells grown on YPD plates at 28°C and 37°C.

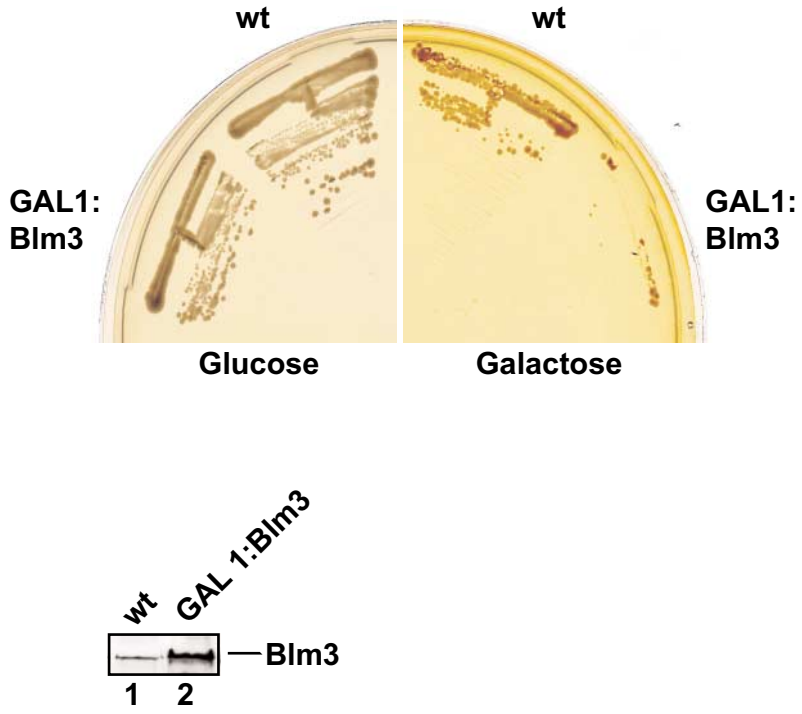

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Figure S1C

(C) Galactose-induced overexpression of Blm3 inhibits cell growth. To express Blm3 behind the GAL1 promoter, the endogenous BLM3 promoter (-60bp referred to ATG) was chromosomally disrupted by the GAL1 promoter. This was achieved by chromosomal integration of LEU2-GAL1 flanked by homologous sequences of the BLM3 promoter region.
On galactose-containing media wild type cells expressing Blm3 behind the GAL1 promoter were growth-inhibited compared to those expressing Blm3 behind the endogenous promoter (upper panels). Overexpression of Blm3 was verified by Western blot analysis using anti-Blm3 antibodies (lower panel; wild type cells expressing Blm3 behind the endogenous promoter (lane 1) and behind the GAL1 promoter (lane 2)). Overexpression of Blm3 did not cause an accumulation of precursor complexes (not shown) indicating that the n-CP rather than Blm3 are limiting in late maturation processes.

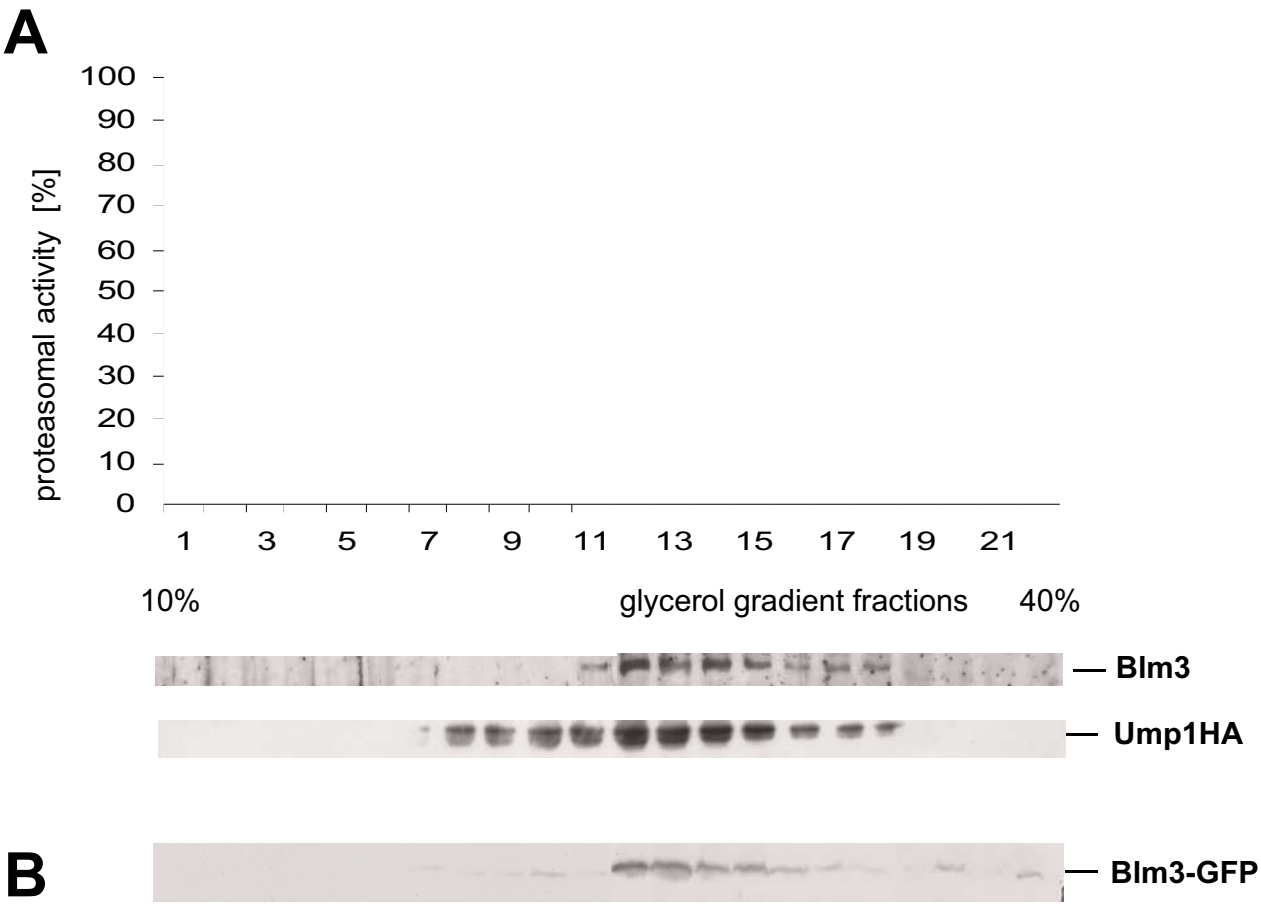

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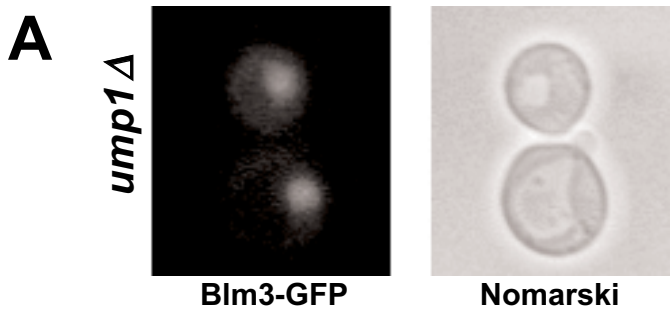
Figure S2

Figure S2. Blm3 co-migrates with Ump1 in density gradient ultracentrifugation. (A) Total extracts of wild type cells expressing Ump1-HA instead of the endogenous protein were subjected to 10-40% glycerol gradient ultracentrifugation as previously described (Enenkel et al., 1998). Fractions were collected from top to bottom and assayed for peptide cleavage activity against Cbz-Leu-Leu-Glu-b-naphthylamide (upper panel). Protein samples of each fraction were run on SDS-PAGE, blotted and probed for Blm3 and Ump1-HA. (B) Extracts of wild type cells expressing GFP-tagged Blm3 were subjected to 10 - 40% glycerol gradient ultracentrifugation as described above and probed for GFP-tagged Blm3.



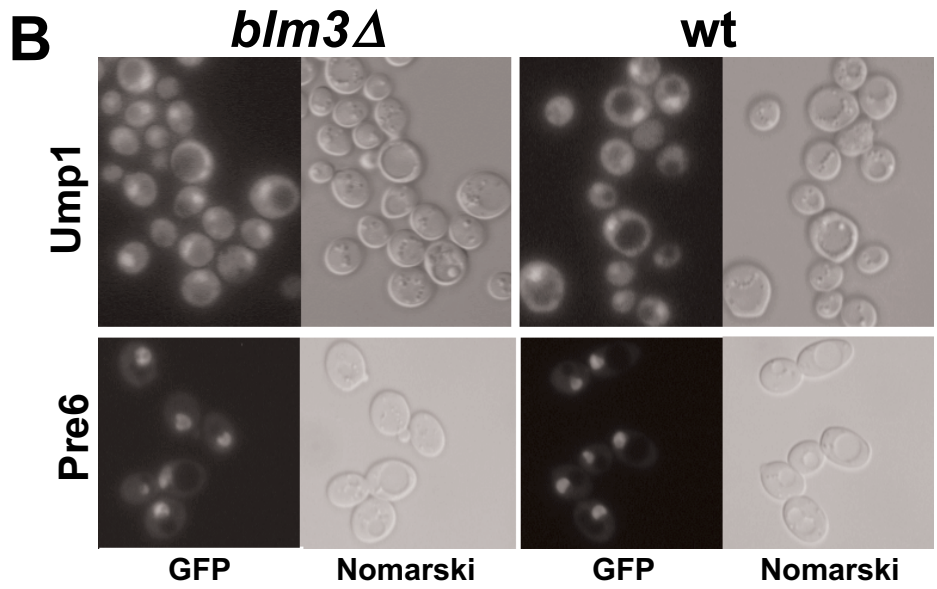


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Figure S3

Figure S3. (A) GFP-labelled Blm3 is nuclear in ump1D cells expressing Blm3-GFP instead of the endogenous protein. Cells were monitored by direct fluorescence microscopy using a GFP filter (left panel) and Nomarski optics (right panel). Precursor complexes were found to be predominantly nuclear in ump1D cells and to be associated with Blm3 (not shown). (B) GFP-labelled Ump1 and Pre6 (a4) are mainly nuclear in blm3D cells. blm3D (left panels) and isogenic wild type cells (right panels) expressing either GFP-tagged Ump1 or Pre6 (a4) were grown to logarythmic phase at 28°C and shifted to 37°C for 4 hours (left panel, GFP filter; right panel, Nomarski optics). Homologous recombination techniques were used to replace proteasomal proteins by GFP-tagged versions in blm3D and ump1D cells (Enenkel et al., 1998).

Table I: Inhibitory effect of Blm3 on m-CP activity

Substrate	Proteasomal activity [%±10]
Z-Gly-Gly-Leu-AMC	114
Succ-Leu-Leu-Val-Tyr-AMC	36
Z-Leu-Leu-Glu-βNA	79
Lactacystin (LLVY))	0
Regulatory 19S particle (LLVY)	250

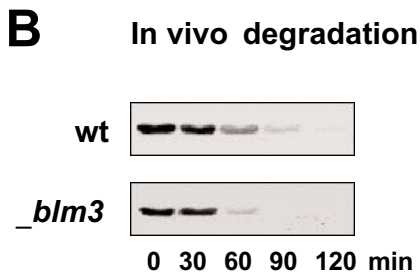

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Figure S4. Inhibitory effect of Blm3 on m-CP activity. (A) SDS-PAGE followed by Coomassie blue staining of purified Blm3. Blm3 was dissociated from affinity-purified proteasomal complexes by 600 mM NaCl and transferred into assay buffer.
Table I: Proteasomal activity was measured in 20 mM Tris/HCl pH 7.8, 5 mM MgCl2, 10 mM KCl, 1 mM DTT for 30 min at 37°C using 100 µM chromogenic substrates Z-LLE-b-naphthylamide (NA), Z-GGL-7-amido-4-methylcoumarin (AMC) and Succ-LLVY-AMC (Ustrell et al., 2002). All measurements were referred to the value of the m-CP without Blm3 (100 %). Complete inhibition of the m-CP was achieved by 50 µM lactacystin. CP and Blm3 were incubated in a molar ratio of 1:10 (70 fmol m-CP). RP was purified (Saeki, Y., Toh-e, A. and Yokosawa, H. (2000) Rapid Isolation and Characterization of the Yeast Proteasome Regulatory Complex. Biochem. Biophys. Res. Commun., 273, 509-515) and added in equimolar ratio. Purified Blm3 had no proteasomal peptide cleavage activity. (B) In vivo degradation of the proteasomal substrate fructose-bisphosphatase (FBPase) in wild type and blm3D cells as analyzed by cycloheximide chase experiments (Horak, J., Regelmann, J., Wolf, D. H. (2002) Two distinct proteolytic systems responsible for glucose-induced degradation of fructose-1,6-bisphosphatase and the Gal2p transporter in the yeast Saccharomyces cerevisiae share the same protein components of the glucose signaling pathway. J Biol Chem 277(10): 8248-8254). We thank the lab members of Dieter Wolf (University of Stuttgart) for support in this experiment.